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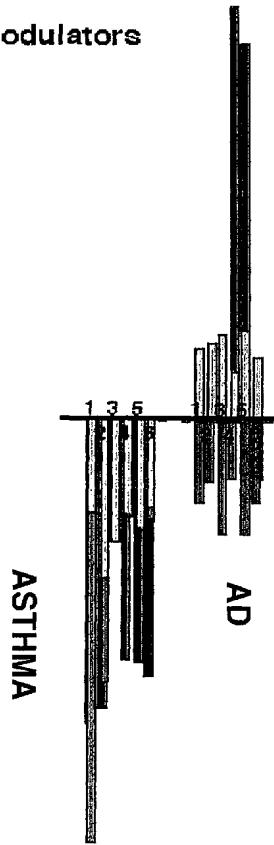
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[Continued on next page]

(54) Title: METHOD FOR DIAGNOSING ALLERGIC DISEASES

Group 3: Tissue modulators

1. Amphiregulin
2. Betacellulin
3. Cripto
4. erbB1
5. erbB3
6. TGF- α



(57) Abstract: mRNA of activated lymphocytes such as CD4+ T cells allows differential diagnosis of allergic diseases. The CD4+ T cells are isolated and stimulated under defined conditions in vitro. Subsequently, mRNA is subjected to multigene analysis such as DNA arrays. Expression profiling images, such as gene expression profiles, can be created, which allow on the basis of the activated T cell mRNA the prediction of certain phenotypes such as asthma or atopic dermatitis.

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Method for diagnosing allergic diseases

The present invention relates to methods for diagnosing allergic diseases and to screening assays for evaluating the 5 allergenic and/or anti-allergical potential of analyte substances using expression profiling images of activated lymphocytes or monocytes/macrophages.

A list of the cited references with full bibliographic data 10 can be found at the end of this specification.

Diagnosis of allergy and allergic asthma is of critical importance for disease management, research, epidemiology and development of specific therapies. Currently diagnosis 15 of allergic diseases is limited to a number of proteins such as serum IgE which suggests but not proofs allergic reactions. Only the reaction against a specific antigen defines the presence of an allergic disease, making the IgE test to the most valuable test in allergy diagnosis (1, 2).

20 However the antigen is in many cases unknown, unless numerous and painful skin tests reveal an allergen. With serum IgE reacting against the allergen the definition for an allergic disease is fulfilled. But even the knowledge of 25 an allergen does not allow conclusion of the immunological status of the patient or prediction of the future development of the disease. Diagnostic routine allows (in clinics with larger diagnosis facilities) the analysis of surface markers by FACS® routine, which is expensive and 30 restricted to the analysis of only a few genes in parallel.

The design of new multigenic diagnostic systems is of critical importance for future management of multigenic diseases like allergy and asthma (3). These systems bear the possibility to: (A) define allergy with great reliability
5 without knowing the antigen, (B) to define subclasses of the disease which allow better treatment and design of new (subgroup specific) drugs, (C) to define risk-groups for anaphylaxis or other complications, (D) to define patients which might be susceptible for immunotherapy, (E) to
10 identify patients which will develop relative steroid resistance, (F) to identify the pathogenesis-causing genes and (G) to develop treatment strategies against these genes.

High throughput methods are now available which would allow
15 the analysis of many expressed genes, however, it is not clear which cellular targets are suitable and which genes or gene products to be screened serve as reliable candidates for the desired purpose. For lung disease bronchial lavage fluids are reasonable targets which require invasive
20 techniques not suitable for many physicians. In case of atopic dermatitis (AD), skin biopsies are performed which allow only histological analysis with limited conclusions.

Blood is for diagnostic procedures the best choice since it
25 can be collected with least invasive methods. The unseparated peripheral blood lymphocytes are in most cases subjected to variation of cell numbers even in healthy subjects and are therefore not of any help. T lymphocytes are known to be the cells, which react in the first line
30 against the allergens and are therefore believed to induce an immune response which may be associated with a disease, by helping B cells to produce IgE. However these cells were

so far not been considered for diagnostic purposes, since the frequency of allergen specific in blood is very low (>0.1%; (4)). Even within skin lesions the frequency of a given allergen such as house dust mite is just 0.4% to 2.7%
5 of all other T cells (5).

Therefore no attempt has been made so far to use, for example, the CD4+ T cell population, since changes of T cell gene expression are only expected in cells reacting against
10 an allergen and not those which are allergen unreactive (>99% of the blood cells).

The use of T lymphocytes in proliferation assays allows the detection of a specific reaction of allergen reactive cells
15 which is measurable following a week of in vitro cultivation (6, 7), and can be used to identify allergens of a given patient. These measurements, however, do not provide more information than any allergen-skin test, i.e. reactivity of a T cell against a given antigen.

20 Thus, an object of the invention is to provide a simple and reliable method for diagnosing allergic disease(s), even without knowing any specific allergen.

25 A further object of the present invention is to provide screening assays to evaluate the allergenic and/or anti-allergic potential of an analyte substance.

These and other objects and features of the invention will
30 be apparent from the description, drawings, and claims,
which follow.

The invention provides a method for diagnosing the presence of or predisposition for (a) allergic disease(s) using expression profiling images comprising the steps of:

- 5 (a) in vitro activating lymphocytes or monocytes/macrophages obtained from whole blood of an individual to be diagnosed for the presence of or predisposition for (a) specific allergic disease(s),
- 10 (b) providing ligands selected from the group consisting of mRNA, oligonucleotides, cDNAs, proteins or functional fragments thereof, isolated or generated from said lymphocytes or monocytes/macrophages,
- 15 (c) contacting said ligands with a set of immobilized receptors selected from the group consisting of oligonucleotide or cDNA probes and antibodies or functional fragments thereof, specifically representing a respective set of genes coding for gene products selected
20 from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, TGF- α , ephrin-A1, ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4,
25 EphB6, BDNF CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R, ICAM-1, Angiogenin, VEGF, VEGF-8, VEGF-D, CD34, SLAM, eolakin-2, IL-8, MIP-1d, MCP-4, MDC, midkine, TARC, Flt-3 ligand, G-CSF, GM-CSF, c-kit ligand, leptin, oncostatin M, osteopontin, PP14, SARP-1, follistatin, IGF binding protein, IGF binding protein, IGF binding protein, Epo R, G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS, MMP-12, inhibin A (a
30 G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS, MMP-12, inhibin A (a

subunit), 4-1BB, CD30, FasL, TRAIL R2, GM-CSF Rb, IFN-a/b Rb, IGF-I R, c-kit, M-CSF R, FGF acidic, FGF-3, FGF-4, FGF R2, FGF R3, integrin-a5, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-11, IL-1 RII, IL-2 Ra, IL-2 Rb, IL-2Rg, GADPH, and allelic variants and mutants thereof, involved with the presence of or predisposition for (a) specific allergic disease(s) to be diagnosed, and qualitatively and quantitatively detecting the presence of bound ligand/receptor complexes to obtain an expression profiling image being representative for the current status of the individual to be diagnosed,

- (d) comparing the expression profiling image obtained in step (c) with the expression profiling image(s) of normal individuals and/or with the expression profiling images(s) of individuals having a predisposition for or suffering from (a) specific allergic disease(s) to be diagnosed, and
- (e) excluding or diagnosing (a) suspected allergic disease(s) or a predisposition therefor on the basis of the results of the comparison obtained in step (d).

According to a preferred embodiment, the gene products referred to in step (c) above are selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2,

neuropilin-1, NGF R, GADPH; and allelic variants or mutants thereof.

The selection of suitable candidate genes is listed in Fig.
5 8.

An advantage of the inventive method is that differential diagnosis of different allergic diseases is possible on the basis of the respective different expression profiling
10 images in vitro, i.e. outside the body. Moreover, in a further embodiment of the inventive method useful as a screening assay potential allergenic substances or anti-allergical substances may be identified.

15 In order to generate a statistically significant imaging, selection of at least 3 to 5 of the defined receptors may be sufficient. It might, however, be possible that a specific allergic disease requires more than 5 genes.

20 The step of in vitro activating the cells is important. When, for example, step (c) is performed by using ligands derived from cells not being in vitro activated before, signals, e.g. on an array, were inconsistent in quality or even absent except of a few housekeeping genes (Fig. 1a,
25 housekeeping genes on the right side). This effect possibly reflects different activation conditions of individual patients. In contrast, in vitro activation enhanced signals on the array, for example a membrane, surprisingly also of genes which are not regulated by anti-CD3/CD28 such as
30 integrins. It appears that anti-CD3/CD28 stimulation enhances the allover transcription machinery, resulting in more mRNA transcripts. The T cell stimulation step therefore

contributes to the normalization of the activation status of the cells and in turn for the standardization of the diagnostic procedure which is important for intra-patient comparisons.

5

Further advantageous and/or preferred embodiments of the invention are subject-matter of the further independent claims and the subclaims.

- 10 In one embodiment of the invention in step (a) of the method said lymphocytes are selected from the group consisting of CD4+ T cells and CD8+ T cells. Using CD8+ T cells may be preferred, in particular when lung diseases are initiated by viral infections, or monocytes might be used for patients
15 with additional inflammatory diseases. It should further be noted that the activation or stimulation in step (a) of the inventive method can also be performed in full, unseparated blood prior to the separation of the cells. The latter modification allows automatic procedures, which will be
20 critical for controlling the costs of the diagnostic procedure.

In a further embodiment of the invention in step (a) of the method said lymphocytes or monocytes/macrophages are activated by exposing the cells to mitogens such as anti-CD3 and/or anti-CD28 antibodies; or phorbol 12-myristate 13-acetate and ionomycin; or phytohemagglutinin and ionomycin; or staphylococcal enterotoxin B; or lipopolysaccharid; or a combination thereof. The anti-CD3 and/or anti-CD28 antibodies may be, for example, monoclonal antibodies, which optionally may be plate-bound or crosslinked.

In yet a further embodiment of the invention in step (b) of the method the ligand is for example labelled with one or more label(s) selected from the group consisting of colored, fluorescent, bioluminescent, chemoluminescent, phosphorescent or radioactive labels, an enzyme, an antibody or a functional fragment or derivative thereof, a protein A/gold based system, a biotin/avidin/streptavidin based system or an enzyme electrode based system is/are used.

10 In a preferred embodiment of the inventive method the allergic disease(s) or predisposition(s) to be diagnosed is/are selected from the group consisting of allergic asthma, allergic rhinitis, food allergies, anaphylactic shock risk, atopic dermatitis (AD), immediate-type allergic reactions, and insect allergies.

More preferably the allergic disease(s) or predisposition(s) to be diagnosed is/are allergic asthma and/or atopic dermatitis (AD). Figure 8 shows the relevance of certain genes being up- or downregulated in these two indications. By excluding allergic asthma it is possible to diagnose non-allergic asthma, which differential diagnosis is of clinical importance.

25 Preferably, the set of receptors employed according to the invention is selected from oligonucleotide or cDNA probes having a sequence defined by, or correlated to, or derived from the group of genes consisting of candidate genes indicated in the following table:

Table 1

Gene (coding for)	Start- position	Length	Accession- number	Intensity
EpCAM	1259	40	X13425	+
DNAM	2369	40	U56102	++
Endoglin	1878	40	X72012	+
Flt-3/Flk-2 R	2367	40	U02687	++
c-met	4305	40	X54559	+
MPL R	1854	40	M90102	+
MSP R	3950	40	X70040	+
IL-18 R	2947	40	U43672	+
Tie-2	2618	42	L06139	++
Fractalkine	216	40	U91835	++
HCC-4	988	40	U91746	++
I-309	427	40	M57502	++
Agouti- related Transcript	720	40	U88063	+++
PREF-1	1395	40	U15979	++
SARP-3	1108	40	AF017988	++
Urokinase R	120	40	Z46797	++
MMP-15	559	40	D86331	++
EBAF	1847	42	U81523	++
TRAIL R2	2922	40	AF016266	+
Amphiregulin	907	40	M30704	+
Betacellulin	1029		NM_001729	+
Cripto	1034		X14253	++
erbB1	4253	40	X00588	++
erbB3	4445	40	M29366	++
Ephrin-A1	929	40	M57730	+++

Ephrin-A2	197	40	AJ007292	+++
EphA1	2598	40	M18391	+
EphA2	2920	40	M59371	+
EphA3	2058	40	M83941	+
EphA4	2443	42	L36645	+
EphB4	3163	40	U07695	+++
EphB6	3276	41	D83492	+++
BDNF	812		X91251	+
CNTF	682	42	X60542	+
GDNF	379	40	L19063	+
GFR□□	942	40	U97144	++
GFR□2	438	40	AF002700	++
Neuropilin-1	2490	40	AF018956	++
NGF R	2885	40	M14764	+++
GADPH	516	40		

, wherein each oligonucleotide or cDNA probe is obtainable on the basis of structural information mediated by the sequence data provided by the respective accession number 5 set out for each candidate gene.

The intensities indicate upregulation or downregulation of the respective gene based on the deviation from standard levels characteristic for a normal's status.

10

For example, suitable sequences which may be used for oligonucleotide design may be downloaded from the NCBI GenBank (<http://www.ncbi.nlm.gov/GenBank/index.html>) using the Accession number listed in Figure 8. All accession numbers give access to mRNA sequences. The oligonucleotide position (see Figure 8) is selected using the Vector NTI 4.0 software (Informix, Gaithersburg, MD, USA) using following

settings for the search of hybridization probes: size of oligonucleotide probe 40 ± 2 ; melting temperature of $T_m = 75 \pm 3$ °C and a GC content of 45-60%.

5 In Figure 8 these genes are roughly classified into 5 functional groups.

In another embodiment of the invention in step (c) of the method said immobilized receptors are located on a surface,
10 and may optionally form a patterned array thereon.

In another embodiment of the invention said array provides
within each such area distinct detection areas with at least
one receptor immobilized thereon being representative for
15 the presence of or predisposition for (a) allergic
disease(s). For example it is possible to diagnose non-
allergic asthma by excluding allergic asthma.

As already outlined before the allergic disease(s) to be
20 diagnosed is/are allergic asthma and/or atopic dermatitis
(AD).

In the case of allergic asthma the preferred and immobilized
receptors specifically represent genes coding for gene
25 products selected from the group consisting of Endoglin,
Flt-3/Flk-2 R, c-met, MPL R, IL-18 R, Fractalkine, HCC-4, I-
309, PREF-1, SARP-3, Urokinase R, EBAF, Amphiregulin,
Betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2,
EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF,
30 GFR α 1, GFR α 2, Neuropilin-1, NGF R and allelic variants or
mutants thereof.

In the case of atopic dermatitis (AD) the preferred and immobilized receptors specifically represent genes coding for gene products selected from the group consisting of EpCAM, DNAM, Flt-3/Flik-2 R, c-met, MSP R, IL-18 R, Tie-2, 5 HCC-4, I-309, Agouti-Related Transcript, SARP-3, Urokinase R, MMP-15, EBAF, Trail R2, Amphiregulin, Betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6 and allelic variants or mutants thereof.

10

In a further aspect the invention provides a screening assay for evaluating the allergenic and/or anti-allergical potential of (an) analyte substance(s) using expression profiling images comprising the steps of:

15

(a) in vitro activating lymphocytes or monocytes/macrophages, preferably being standardized to represent a baseline expression profiling image characteristic for a normal's status,

20

(b) incubating said activated lymphocytes or monocytes/macrophages with (an) analyte substance(s) to be evaluated for its (their) allergenic and/or anti-allergical potential,

25

(c) providing ligands selected from the group consisting of mRNA, oligonucleotides, cDNAs, proteins or functional fragments thereof, isolated or generated from said lymphocytes or monocytes/macrophages,

30

(d) contacting said ligands with a set of preferred and immobilized receptors selected from the group consisting of oligonucleotide or cDNA probes and antibodies or

functional fragments thereof, specifically representing a respective set of genes coding for gene products selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, TGF- α , ephrin-A1, ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R, ICAM-1, Angiogenin, VEGF, VEGF-8, VEGF-D, CD34, SLAM, eolakin-2, IL-8, MIP-1d, MCP-4, MDC, midkine, TARC, Flt-3 ligand, G-CSF, GM-CSF, c-kit ligand, leptin, oncostatin M, osteopontin, PP14, SARP-1, follistatin, IGF binding protein, IGF binding protein, IGF binding protein, Epo R, G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS, MMP-12, inhibin A (a subunit), 4-1BB, CD30, FasL, TRAIL R2, GM-CSF Rb, IFN-a/b Rb, IGF-I R, c-kit, M-CSF R, FGF acidic, FGF-3, FGF-4, FGF R2, FGF R3, integrin-a5, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-11, IL-1 RII, IL-2 Ra, IL-2 Rb, IL-2R γ , GADPH, and allelic variants or mutants thereof, involved with the presence of or predisposition for (a) specific allergic disease(s), and qualitatively and quantitatively detecting the presence of bound ligand/receptor-complexes to obtain an expression profiling image being representative for the allergenic and/or anti-allergical potential of the analyte substance(s) to be evaluated,

- (e) evaluating the allergenic and/or anti-allergical potential of the analyte substance(s) by comparing the expression profiling image obtained in step (d) with the expression profiling image generated from normal

individuals, or preferably by directly analyzing the expression profiling image obtained in step (d).

According to a preferred embodiment of the invention in step 5 (d) of the screening assay said gene products are selected from the group consisting of EpcAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, 10 betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, neuropilin-1, NGF R, GADPH; and allelic variants or mutants thereof.

15 The "risk degree" of an allergenic substance can be concluded by comparison of the expression profiles with substances of known risk (such as diesel exhaust, Aspergillus allergens etc.).

20 For example, food allergies, anaphylactic shock risk patients, immediate-type allergic reactions, insect allergies, allergies against environmental pollutants or medicaments may be easily detected.

25 The current allergic status of a patient, for example treated with an anti-allergically active substance, may be monitored easily with the inventive screening assay. This allows a prompt adaptation of the treating regime (for example dosing, type of administration) to the current 30 allergic status. Anti-allergic substances are for example sympathomimetic drugs, histamine antagonists, glucocorticoids, and corticosteroids. Specific examples may be found

in any standard textbook on pharmacology, cf. for example Goodman & Gilman's, "The Pharmacological Basis of Therapeutics", Vols. and 2, 8th edition, McGraw-Hill International Editions, 1992, pages 217, 587, 1455 and 5 1575/76 the disclosure content of which is herein incorporated by reference.

Of particular clinical importance is that patients with relative steroid resistance or patients that will develop 10 such resistance may be identified easily with the inventive screening assay.

In another aspect the invention provides a diagnostic tool suitable for use in the inventive methods comprising on its 15 surface a set of immobilized receptors selected from the group consisting of oligonucleotide or cDNA probes and antibodies or functional fragments thereof, capable of specifically binding ligands selected from the group consisting of mRNA, oligonucleotides, cDNAs, proteins or functional fragments thereof, representing a respective set 20 of genes coding for gene products selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP- 25 15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, TGF- α , ephrin-A1, ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R, ICAM-1, Angiogenin, VEGF, VEGF-8, VEGF-D, CD34, SLAM, eolakin-2, IL-8, MIP-1d, MCP-4, MDC, midkine, 30 TARC, Flt-3 ligand, G-CSF, GM-CSF, c-kit ligand, leptin, oncostatin M, osteopontin, PP14, SARP-1, follistatin, IGF binding protein, IGF binding protein, IGF binding protein,

Epo R, G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS, MMP-12, inhibin A (a subunit), 4-1BB, CD30, FasL, TRAIL R2, GM-CSF Rb, IFN-a/b Rb, IGF-I R, c-kit, M-CSF R, FGF acidic, FGF-3, FGF-4, FGF R2, FGF R3, integrin-a5, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, 5 IL-10, IL-11, IL-1 RII, IL-2 Ra, IL-2 Rb, IL-2Rg, GADPH, and allelic variants or mutants thereof, involved with (a) specific allergic disease(s) or a predispositon therefor to be diagnosed.

10 According to a preferred embodiment of the inventive diagnostic tool said gene products are selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, 15 MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, neuropilin-1, NGF R, GADPH; and allelic variants or mutants thereof.

20 Optionally, said immobilized receptors may form a patterned array on the surface. If, for example fluorescent nucleotides are used the surface may be made of glass or any other solid, non-fluorescent carrier.

25 As already outlined before the array may provide distinct detection areas with at least one receptor immobilized within each such area.

30 According to a preferred embodiment of the invention the diagnostic tool is suitable to diagnose allergic asthma, and wherein the immobilized receptors specifically represent at

- least one gene coding for at least one gene product selected from the group consisting of Endoglin, Flt-3/Flk-2 R, c-met, MPL R, IL-18 R, Fractalkine, HCC-4, I-309, PREF-1, SARP-3, Urokinase R, EBAF, Amphiregulin, Betacellulin, Cripto,
5 erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R, GADPH, and allelic variants or mutants thereof.
- 10 According to another preferred embodiment of the invention the diagnostic tool is suitable to diagnose atopic dermatitis, and wherein the immobilized receptors specifically represent at least one gene coding for at least one gene product selected from the group consisting of
15 EpCAM, DNAM, Flt-3/Flk-2 R, c-met, MSP R, IL-18 R, Tie-2, HCC-4, I-309, Agouti-Related Transcript, SARP-3, Urokinase R, MMP-15, EBAF, Trail R2, Amphiregulin, Betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, and allelic variants or mutants
20 thereof.

The inventive method generally allows the generation of characteristic expression profiling images for diagnosing allergic diseases based both on genes and the respective gene products, i.e. proteins. In a specific embodiment gene expression profiling images or gene expression profiles are used.

As already mentioned above, the present invention proposes
30 the use of mRNA isolated from for example CD4+ T cells or monocytes/macrophages activated in vitro, for example for 6h, as a basis for multigene analysis for differential

diagnosis of allergic and asthmatic diseases. Here it is demonstrated for the first time that, for example, hybridization techniques are sensitive enough to profile T cell or monocytes/macrophages gene expression and that 5 multigene approaches allow on the basis of activated CD4+ T cell or monocytes/macrophages mRNA the differentiation between clinically different allergic diseases, such as allergic asthma and atopic dermatitis (AD) by gene expression profiles.

10

In the following the invention is described in more detail, however, without any limitation, with respect to complementary nucleic acids or fragments thereof as ligand/receptors pairs.

15

In practicing the invention, after steps (a) and (b) of the inventive method each gene, i.e. labelled cDNA derived therefrom, involved with the presence of or predisposition for (a) specific allergic disease(s) to be diagnosed is 20 spotted in duplicates onto, for example, an array membrane with respective immobilized oligonucleotide probes capable of specifically hybridizing with the respective gene, i.e. labelled cDNA derived therefrom, and the mean of both measurements is used in subsequent evaluations. Differences 25 in gene expression are accepted as different when the signals from patient material are at least two fold lower or higher than those from healthy volunteers. This threshold is commonly accepted for the evaluation of expression arrays. All genes are corrected against housekeeping genes to 30 compensate minor differences in cell load of each sample.

Fold differences are calculated for each gene and since the order and scale of each patient is the same an individual profile for each patient results. These profiles are now overlaid (middle panel of Figure 2), for example for all 5 atopic dermatitis (AD) and for all asthma patients, resulting in an (ideal) asthma or atopic dermatitis profile. This profile is defined by the probability value p , which will be defined for each individual gene or gene groups in correlation (Spearman Rank Test) with clinical parameters of 10 asthma or atopic dermatitis. For normal profiles healthy volunteers are used.

The inventive method is in addition supported by statistical analysis, which defines statistical confidence intervals, 15 which link for example a 5 fold decrease in gene x with 10% confidence to phenotype y; a 7 fold decrease in gene x with 50% confidence to phenotype y. Again this confidence is defined on the basis of probability value P (precise formula given in (8)). Even if statistical correlation with a given 20 phenotype is relatively low using the inventive method, the practically unlimited number of genes and their statistical confidence will be in sum 99%, since the p values are added for the confidence intervals (8). The number of genes necessary for the method of the invention depends finally on 25 the phenotype to be described and its extend of correlation with each gene. For example 3 to 5 genes from each group in the table of Figure 8 may be sufficient to obtain a statistically significant normal or allergic profile.

30 The foregoing and other objects of the invention, the various features thereof, as well as the invention itself,

may be more fully understood from the following description, when read together with the accompanying drawings, in which:

Figure 1 shows autoradiographs of labelled mRNA from not
5 activated and activated CD4+ cells.

Figures 2 shows gene expression profiles of individual patients suffering from allergic asthma or atopic dermatitis. Three blocks are representing three individual
10 patients. Of note these bars represent changes in comparison to healthy volunteers. Within each patient block are 5 groups which contain genes which share certain functions. Each group is depicted separately in Figure 3-7 including the gene designation. On the right next to the individual
15 patients are overlays of the patient groups shown. These overlays demonstrate that some genes are exclusive marker for atopic dermatitis or asthma respectively whereas other genes are "misregulated" both in atopic dermatitis and asthma.

20
Figure 3 shows surface genes (group 1) which are likely to be expressed during the T cell activation process. The left panel shows the changes in atopic dermatitis patients (AD) and the right panel the asthma patients. The bars represent
25 changes in fold expression. A bar upward from the black lines indicates an increase a bar downwards a decrease of expression. Epcam (lane 1) was in all three AD patients decreased, whereas asthma patients show either a weak decrease or even an increased expression. DNAM (lane 2) was increased in AD patients except in one patient, similarly to asthma patients, possibly indicating a common allergy gene.
30 Endoglin (lane 3) was decreased in all asthma patients

whereas AD patients showed increased expression in two cases and only a minor decrease in one case. The ladder patient is possibly predisposed for developing asthma. Flt-3/Flk-2 R (4) was always found to be increased in AD patients whereas 5 asthma patients showed decreased expression except in one case. C-met (lane 5) was again decreased in all asthma patients and increased in AD patients with exception of the putative "asthma predisposed" patient. MPL R (lane 6) behaved as described for C-met. MSP-R (lane 7) was dramatically increased in two asthma patients with the exception of one patient who showed a minor decrease, in contrast two AD patients showed a decrease in gene expression and only one patient showed a minor increase. The expression of the IL-18-R (lane 8) was more heterogeneously 10 expressed, however increases observed both in asthma and also a single but dramatic decrease in an AD was observed. Tie-2 (lane 9) was unchanged in asthma patients but dramatically up regulated in two AD patients and rather to a minor degree decreased in another AD patient.

20 Figure 4 shows cytokines (group 2) which are important for cross talk between lymphocytes and other cells. The left panel shows the changes in atopic dermatitis patients (AD) and the right panel the asthma patients. The bars represent 25 changes in fold expression. A bar upward from the black lines indicates an increase a bar downwards a decrease of expression. Fractalkine (lane 1) was always found to be decreased in asthma patients and increased in two out of three AD patients. The AD patient which showed a decrease is 30 identical with the putative "asthma predisposed" AD patient described in Figure 3. HCC-4 (was decreased in all asthma and all AD patients, possibly representing a common allergy

marker, which is known to be regulated by IL-10, a suppressive cytokine. I-309 (lane 3) behaves as described for Fractalkine. The Agouti-related transcript was decreased in two and increased to a minor degree in one asthma patient: in AD patients changes occurred in a lower amplitude but reverse (increased) in comparison to the asthma patients. PREF-1 (lane 5) showed a dramatic increase in a single asthma patient and a decrease in the other two patients: in AD patients this gene behaved as described for Fractalkine. SARP-3 (lane 6) decreased in all asthma patients whereas expression was unchanged in AD patients except of one case where a minor increase was observed. The urokinase receptor was mostly upregulated both in asthma and AD, indicating an allergy related misregulation of this gene. MMP-15 (lane 8) expression is comparable with the urokinase receptor described above. EBAF (lane 9) showed only minor changes in AD patients, whereas downregulation was detected in all three asthma patients. TRAIL R2 (lane 10) was upregulated in all three AD patients and also in two asthma patients. Only one asthma patient showed a minor decrease in TRAIL R2 expression.

Figure 5 shows factors important for tissue modeling (group 3). The left panel shows the changes in atopic dermatitis patients (AD) and the right panel the asthma patients. The bars represent changes in fold expression. A bar upward from the black lines indicates an increase a bar downwards a decrease of expression. Amphiregulin (lane 1) was downregulated in all three asthma patients. A mild increase in Amphiregulin expression was only observed in one AD patient and a decrease in the patient described above with the putative "asthma predisposition". Betacellulin (lane 2)

showed the same patterns as Amphiregulin. Cripto (lane 3) showed a decrease in two asthma patients and behaved for the AD patients as described for the preceding genes. ErbB1 (lane 4), ErbB3 (lane 5) and TGF-alpha (lane 6) were in all three asthma patients decreased and increased (in particular ErbB1 & 3) in the AD patients with the exception of the putative "asthma predisposition" patient.

Figure 6 shows factors (group 4) related to neurological biology which are however not yet functionally described. The left panel shows the changes in atopic dermatitis patients (AD) and the right panel the asthma patients. The bars represent changes in fold expression. A bar upward from the black lines indicates an increase a bar downwards a decrease of expression. Strikingly, all of the genes (lanes 1-8; Ephrin-A1, Ephrin-A2, EphA1-4 and EphB4 & 6) were decreased in all asthma patients with the exception EphA1 (lane 3) and EpHA3 (lane 5), which was increased in one asthma patient. As in previous figures, the putative "asthma predisposition" patient showed also a decrease in all genes. In contrast, Ephrin-A1, A2 and EphA1-3 (lanes 1-5) were increased in all other AD patients. EphA4, B4 and B6 (lanes 6-8) were decreased in one AD patient and decreased in the third one.

Figure 7 shows neurotrophic factors (group 5) which allow the T cells the communication with neuronal cells. The left panel shows the changes in atopic dermatitis patients (AD) and the right panel the asthma patients. The bars represent changes in fold expression. A bar upward from the black lines indicates an increase a bar downwards a decrease of expression. CNTF (lane 1) was increased in two patients

decreased to a minor degree in one AD patient. In Asthma two patients with strong increase and one patient with a minor decrease were observed. GDNF (lane 2) was increased in one patient and decreased in two AD patients however the greater 5 amplitude was seen in the case of increased expression. Also asthma patients showed only increased expression of this gene with a great amplitude. GFRa1 (lane 3) was increased and decreased in both disease, but the greatest amplitude was seen in the increased expression of AD patients and the 10 greatest in decrease in the asthma patient. GFRa2 (lane 4) behaved as BDNF in AD patients, whereas all three asthma patients showed an increase in gene expression. Neuropilin-1 (lane 5) was dramatically increased in two AD patients and decreased to a minor degree in another AD patient. Among 15 asthma patients Neuropilin was increased in one patient and decreased in another patient and unchanged in a third one. NGF R (lane 6) Increased in two AD patients to a minor degree and decreased to the same extend in another one. Although one asthma patient was observed with a mild 20 increase in NGF R expression, the two other patients showed a marked decrease in NGF R expression. Of not, the AD patient with "asthma predisposition" was not observed to be asthma-like in this panel, indicating that genes of this group might correlate with acute clinical symptoms.

25
Figure 8 shows a list of genes involved with allergic asthma or atopic dermatitis (AD). The genes are clustered as described in figures 3-7. Further, positions of sequences for oligonucleotide probes (with data bank accession numbers 30 for the respective genes) are given. The oligonucleotides in a size of 40 ± 2 base pairs were selected on the basis of a

common melting temperature (T_m 75 ± 3 °C, GC content: 45-60%).

In the following the invention is disclosed in more detail
5 with reference to examples and to drawings. However, the described specific forms or preferred embodiments are to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the following description,
10 and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

Particularly, it should be appreciated that in the following
15 description activated CD4+ T cells are used only as a specific example for suitable blood cells, and that allergic asthma and atopic dermatitis are only specific examples for allergic diseases.

20

Examples

To perform diagnosis tests 80 ml blood is taken from the patient using vacuum container containing Heparin (Vacutainer™, Becton Dickinson, Pymouth, UK). For pediatric tests the volume of blood can be downscaled to 5 ml if the RNA is amplified following isolation and prior labelling. CD4+ T cells are separated from other blood cells using either Ficoll (Biocoll separating solution, Biochrom KG, Berlin, Germany) density centrifugation following the instructions of the manufacturer. Purified peripheral blood mononuclear cells (PBMC) are subsequently purified using the anti-CD4 coated magnetic beads (DetachAbead, Dynal, Oslo, Norway).

The purity of the isolated population is verified by FACS® analysis and is always higher than 95%.

Cells are cultured overnight with plate-bound anti-CD3 (1 μ g / ml; OKT3 clone from ATCC, Manassas, VA, USA) and anti-CD28 (2 μ g / ml; 15E8 from CLB, Amsterdam, The Netherlands) monoclonal antibodies. All cultures are carried out in RPMI 1640 supplemented with 1 mM L-glutamine, sodium pyruvate, non-essential amino acids, 5 x 10⁻⁵ μ M 2-mercaptoethanol and 10% heat-inactivated fetal calf serum (all from Life Technologies, Basel, Switzerland).

For RNA analysis 5 x 10⁶ cells of each cell population are lysed with lysis buffer (Rneasy, Qiagen, Hamburg, Germany). Alternatively cells can be directly purified from blood by using the Dynal® beads described above, avoiding the Ficoll® step. There is preliminary evidence that stimulation with plate-bound antibodies can be replaced by mitogenic stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin or phytohemagglutinin (PHA) and ionomycin. As already mentioned above, this stimulation can also be performed in full, unseparated blood prior separation of the cells.

RNA is isolated using the Rneasy® kit (Qiagen, Hamburg, Germany) following the instructions of the supplier and eluted in 100 μ l H₂O. The RNA is precipitated with sodium acetate and Ethanol, washed with 70 % ethanol and resuspended in 11 μ l H₂O.

30

The RNA is mixed with 4 μ l dT12 primers (Microsynth, Bal-gach, Switzerland) and incubated in a thermal cycler at 90° C

for 2 minutes. The temperature is ramped to 42° C in 20 minutes. Following components are added: 6 µl 5× reverse first strand buffer (GIBCO BRL, Basel, Switzerland), 2 µl 0,1 M DTT (Life Technologies, Basel, Switzerland), 20 U 5 RNase inhibitor (Roche, Mannheim, Germany), 1 µl of dCTP, dGTP, dTTP (each 10 mM; Perkin Elmer), 5 µl [α -33P]-dATP (0,4 MBq/µl; Hartmann, Braunschweig, Germany) and 1 µl RNase H-reverse transcriptase (Superscript II, Life Technologies). Alternatively, for example fluorescent nucleotides can be 10 used in combination with e.g. glass arrays or any other solid, non-fluorescent carrier. The mixture is incubated at 42°C for 3 hours and filled up to 100 µl with H₂O. The cDNA is purified using silica-gel columns (QIAquick, Qiagen) and eluted in 100 µl H₂O. Again 1 µl are measured in a 15 scintillation counter to estimate the percentage incorporation of labeled nucleotides in the cDNA.

The oligonucleotide probes and an array may be constructed as described above, e.g. the oligonucleotides listed in 20 Figure 8 can be spotted on nylon membrane: The oligonucleotides are spotted on the membrane using a 96 slot manifold (Schleicher & Schuell, Dassel, Germany). The DNA is denatured in 50 µl 1N NaOH incubated for 5 min at 37°C and subsequently mixed with 12x SSC buffer (1.8 M NaCl, 0.18 25 trisodium citrate). A total amount of 10 ng of each oligonucleotide is added in a volume of 100 µl to allow equal distribution on the membrane and are arranged in duplicates. In the present example, however, a commercially available array membrane is used. One probe from patient 30 blood and one probe from healthy volunteer blood is each hybridized on a human cytokine expression array membrane (R&D Systems, Abingdon, UK). Hybridization in the present

example is performed as described in the human cytokine expression array handbook (R&D). The membranes are washed 20 minutes with 30 ml of a 0,1 × SSPE buffer (SSPE stock is 20x: 5 3.6M NaCl, 0.2 M NaH₂PO₄, 0.02 M EDTA, pH7.7), 1% (w/v) SDS solution. The arrays are exposed 24 hours to imager-screens (Fuji Film, Tokyo, Japan). The screens are analyzed using the FLA 3000 phosphor imager system (Fuji) and evaluated with the AIDA program (Raytest, Urdorf, Switzerland). To compare the intensity of spots containing different 10 membranes housekeeping genes like GAPDH and L19 are normalized.

Out of 375 different genes 100 have been found to be regulated in either asthma or atopic dermatitis patients. 15 Some of the genes with great amplitude are shown in Figure 2. The maximal amplitude detected was 40x, however in most cases the differences did not exceed a factor of 5. Although some genes were disregulated both in asthma and atopic dermatitis, the amplitude was in most cases different. 20 Furthermore, genes with greatest changes were different between atopic dermatitis and asthma (Fig. 2). These results show that the inventive method described here allows the differentiation between allergic diseases.

25 An example of an so-called ATRP (activated CD4+ T cell mRNA profiling system, according to one embodiment of the inventive method) for differential diagnosis of allergic diseases is shown in Figure 2: A set of genes is measured by arrays or other systems. Fold differences are shown for each 30 gene and the order and scale of each patient is the same, resulting in an individual profile for each patient. These profiles are now overlaid (middle panel) for all atopic

dermatitis and for all asthma patients resulting in an (ideal) asthma or atopic dermatitis profile. It should be noted that this phenotype can be clinically more challenging such as revealing bronchial hyperreactivity, steroid resistance or yet unidentified subgroups of allergic disease.

The comparison of atopic dermatitis and asthma is shown in the right panel: Although some genes can be increased or decreased both in asthma or atopic dermatitis, some genes clearly appear only changed in asthma or atopic dermatitis. This comparison is shown in more detail in figures 3-7. Generally it can be concluded that group 1 genes (Fig. 3, surface activation markers) are more frequently increased in AD patients whereas asthma patients showed rather reduced levels. Group 1 genes will give information how active/acute inflammation is going on. For group 2 (Fig. 4) there is generally a higher expression in AD, whereas asthma patients are more suppressed in these genes. Of note HCC-4 is regulated to a lower degree and acts as a marker of reduced tolerogenic capacity of the patient. In contrast TRAIL R2 was increased in both patients reflecting alterations in cell death pathways. Group 3 (Fig. 5) is consistently down-regulated in asthma patients. AD patients with decreased expression levels in this field possibly carry a risk to develop asthma, group 4 genes (Fig. 6) are yet not characterized in their function, but are generally decreased in asthma patients. The same atopic dermatitis patient which showed asthma indicative decreases in group 3 genes is also decreased in group 4 genes, confirming the asthma like profile. Group 5 genes (Fig. 7) are parameters, which are related to nerve growth and activation relevant for pain

diagnosis and for bronchial hyperactivity. A consistent high expression occurs within the asthma patients. The NGF R. is an exception, which was decreased in asthma and increased in AD patients. The complete description of phenotype relation 5 of each gene is listed in the table of Figure 8.

As demonstrated above, mRNA of activated CD4+ T cells allows differential diagnosis of allergic diseases. The CD4+ T cells are isolated and stimulated under defined conditions 10 in vitro. Subsequently, mRNA is subjected to multigene analysis such as DNA arrays. Gene expression profiles can be created, which allow on the basis of the activated T cell mRNA the prediction of certain phenotypes such as asthma or atopic dermatitis.

15

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Claims

1. Method for diagnosing the presence of or predisposition
for (a) allergic disease(s) using expression profiling
5 images comprising the steps of:

(a) in vitro activating lymphocytes or
monocytes/macrophages obtained from whole blood of an
individual to be diagnosed for the presence of or
10 predisposition for (a) specific allergic disease(s),

(b) providing ligands selected from the group consisting
of mRNA, oligonucleotides, cDNAs, proteins or
functional fragments thereof, isolated or generated
15 from said lymphocytes or monocytes/macrophages

(c) contacting said ligands with a set of immobilized
receptors selected from the group consisting of
oligonucleotide or cDNA probes and antibodies or
functional fragments thereof, specifically
representing a respective set of genes coding for
20 gene products selected from the group consisting of
EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R,
MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309,
Agouti-related transcript, PREF-1, SARP-3, Urokinase
25 R, MMP-15, EBAF, TRAIL R2, amphiregulin,
betacellulin, Cripto, erbB1, erbB3, TGF- α , ephrin-A1,
ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6,
BDNF CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R,
30 ICAM-1, Angiogenin, VEGF, VEGF-8, VEGF-D, CD34, SLAM,
eolakin-2, IL-8, MIP-1d, MCP-4, MDC, midkine, TARC,
Flt-3 ligand, G-CSF, GM-CSF, c-kit ligand, leptin,

oncostatin M, osteopontin, PP14, SARP-1, follistatin, IGF binding protein, IGF binding protein, IGF binding protein, Epo R, G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS, MMP-12, inhibin A (a subunit), 4-1BB, CD30, FasL, TRAIL R2, GM-CSF Rb, IFN- α /b Rb, IGF-I R, c-kit, M-CSF R, FGF acidic, FGF-3, FGF-4, FGF R2, FGF R3, integrin- α 5, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-11, IL-1 RII, IL-2 Ra, IL-2 Rb, IL-2Rg, GADPH, and allelic variants or mutants thereof, involved with the presence of or predisposition for (a) specific allergic disease(s) to be diagnosed, and qualitatively and quantitatively detecting the presence of bound ligand/receptor complexes to obtain an expression profiling image being representative for the current status of the individual to be diagnosed,

(d) comparing the expression profiling image obtained in step (c) with the expression profiling image(s) of normal individuals and/or with the expression profiling images(s) of individuals having a predisposition for or suffering from (a) specific allergic disease(s) to be diagnosed, and

(e) excluding or diagnosing (a) suspected allergic disease(s) or a predisposition therefor on the basis of the results of the comparison obtained in step (d).

2. Method according to claim 1, wherein in said gene products are selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related

transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, 5 neuropilin-1, NGF R, GADPH; and allelic variants or mutants thereof.

10 3. Method according to claim 1 or 2, wherein in step (a) said lymphocytes are selected from the group consisting of CD4+ T cells and CD8+ T cells.

15 4. Method according to any of claims 1 to 3, wherein in step (a) said lymphocytes or monocytes/macrophages are activated by exposing the cells to mitogens such as anti-CD3 and/or anti-CD28 antibodies; or phorbol 12-myristate 13-acetate and ionomycin; or phytohemagglutinin and ionomycin; or staphylococcal enterotoxin B; or lipopolysaccharid; or a combination thereof.

20 5. Method according to any one of the preceding claims, wherein the allergic disease(s) or predisposition(s) to be diagnosed is/are selected from the group consisting of allergic asthma, allergic rhinitis, food allergies, anaphylactic shock risk, atopic dermatitis, immediate-type allergic reactions, and insect allergies. 25

6. Method according to claim 5, wherein the allergic disease(s) or predisposition(s) to be diagnosed is/are allergic asthma and/or atopic dermatitis.

7. Method according to any of claims 2 to 6, wherein said set of receptors is selected from oligonucleotide or cDNA probes having a sequence defined by, or correlated to, or derived from the group of genes consisting of candidate genes indicated in the following list:

Gene (coding for)	Startposition	length	Accession No.
EpCAM	1259	40	X13425
DNAM	2369	40	U56102
Endoglin	1878	40	X72012
Flt-3/Flk-2 R	2367	40	U02687
c-met	4305	40	X54559
MPL R	1854	40	M90102
MSP R	3950	40	X70040
IL-18 R	2947	40	U43672
Tie-2	2618	42	L06139
Fractalkine	216	40	U91835
HCC-4	988	40	U91746
I-309	427	40	M57502
Agouti-Related Transcript	720	40	U88063
PREF-1	1395	40	U15979
SARP-3	1108	40	AF017988
Urokinase R	120	40	Z46797
MMP-15	559	40	D86331
EBAF	1847	42	U81523
TRAIL R2	2922	40	AF016266
Amphiregulin	907	40	M30704
Betacellulin	1029		NM_001729
Cripto	1034		X14253

erbB1	4253	40	X00588
erbB3	4445	40	M29366
Ephrin-A1	929	40	M57730
Ephrin-A2	197	40	AJ007292
EphA1	2598	40	M18391
EphA2	2920	40	M59371
EphA3	2058	40	M83941
EphA4	2443	42	L36645
EphB4	3163	40	U07695
EphB6	3276	41	D83492
BDNF	812		X91251
CNTF	682	42	X60542
GDNF	379	40	L19063
GFR□1	942	40	U97144
GFR□2	438	40	AF002700
Neuropilin-1	2490	40	AF018956
NGF R	2885	40	M14764
GADPH	516	40	

wherein each oligonucleotide or cDNA probe is obtainable on the basis of structural information mediated by the sequence data provided by the respective accession number set out for each candidate gene.

- 5 8. Method according to any of the preceding claims, wherein said immobilized receptors are located on a surface.
- 10 9. Method according to claim 8, wherein said immobilized receptors form a patterned array on said surface.

10. Method according to claim 9, wherein said array provides distinct detection areas with at least one receptor immobilized within each such area being representative for the presence of or predisposition for (a) allergic
5 disease(s).

11. Method according to any of the preceding claims, wherein the allergic disease or predisposition to be diagnosed is allergic asthma, and wherein the immobilized receptors
10 specifically represent at least one gene coding for at least one gene product selected from the group consisting of Endoglin, Flt-3/Flk-2 R, c-met, MPL R, IL-18 R, Fractalkine, HCC-4, I-309, PREF-1, SARP-3, Urokinase R, EBAF, Amphiregulin, Betacellulin, Cripto, erbB1, erbB3,
15 Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R, and allelic variants or mutants thereof.

12. Method according to any of claims 1 to 10, wherein the
20 allergic disease or predisposition to be diagnosed is atopic dermatitis, and wherein the immobilized receptors specifically represent at least one gene coding for at least one gene product selected from the group consisting of EpCAM, DNAM, Flt-3/Flk-2 R, c-met, MSP R, IL-18 R, Tie-2, HCC-4, I-309, Agouti-Related Transcript, SARP-3, Urokinase R, MMP-15, EBAF, Trail R2, Amphiregulin, Betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, and allelic
25 variants or mutants thereof.

13. Screening assay for evaluating the allergenic and/or anti-allergical potential of (an) analyte substance(s) using expression profiling images comprising the steps of:

5

(a) in vitro activating lymphocytes or monocytes-macrophages, preferably being standardized to represent a baseline expression profiling image characteristic for a normal's status,

10

(b) incubating said activated lymphocytes or monocytes/macrophages with (an) analyte substance(s) to be evaluated for its (their) allergenic and/or anti-allergical potential,

15

(c) providing ligands selected from the group consisting of mRNA, oligonucleotides, cDNAs, proteins or functional fragments thereof, isolated or generated from said lymphocytes or monocytes/macrophages,

20

(d) contacting said ligands with a set of immobilized receptors selected from the group consisting of oligonucleotide or cDNA probes and antibodies or functional fragments thereof, specifically representing a respective set of genes coding for gene products selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, TGF- α , ephrin-A1, ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6,

BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R,
ICAM-1, Angiogenin, VEGF, VEGF-8, VEGF-D, CD34, SLAM,
eolakin-2, IL-8, MIP-1d, MCP-4, MDC, midkine, TARC,
Flt-3 ligand, G-CSF, GM-CSF, c-kit ligand, leptin,
5 oncostatin M, osteopontin, PP14, SARP-1, follistatin,
IGF binding protein, IGF binding protein, IGF binding
protein, Epo R, G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS,
MMP-12, inhibin A (a subunit), 4-1BB, CD30, FasL,
TRAIL R2, GM-CSF Rb, IFN-a/b Rb, IGF-I R, c-kit, M-
10 CSF R, FGF acidic, FGF-3, FGF-4, FGF R2, FGF R3,
integrin- α 5, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-
15 10, IL-11, IL-1 RII, IL-2 Ra, IL-2 Rb, IL-2Rg, GADPH;
and allelic variants or mutants thereof, involved
with the presence of or predisposition for (a)
15 specific allergic disease(s), and qualitatively and
quantitatively detecting the presence of bound
ligand/receptor-complexes to obtain an expression
profiling image being representative for the
allergenic and/or anti-allergical potential of the
analyte substance(s) to be evaluated,

20 (e) evaluating the allergenic and/or anti-allergical
potential of the analyte substance(s) by comparing
the expression profiling image obtained in step (d)
25 with the expression profiling image generated from
normal individuals, or preferably by directly
analyzing the expression profiling image obtained in
step (d).

30 14. Screening assay according to claim 13 wherein said gene
products are selected from the group consisting of EpCAM,
DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18

R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, 5 EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, neuropilin-1, NGF R, GADPH; and allelic variants or mutants thereof.

15. Diagnostic tool suitable for use in a method according to
10 any one of claims 1 to 12, or in a method according to
claim 13 or 14, comprising on its surface a set of
immobilized receptors selected from the group consisting
of oligonucleotide or cDNA probes and antibodies or
functional fragments thereof, representing a respective
15 set of genes coding for gene products selected from the
group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R,
c-met, MPL R, MSP R, IL-18 R, Tie-2, fractalkine, HCC-4,
I-309, Agouti-related transcript, PREF-1, SARP-3,
Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin,
20 betacellulin, Cripto, erbB1, erbB3, TGF- α , ephrin-A1,
ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6,
BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R,
ICAM-1, Angiogenin, VEGF, VEGF-8, VEGF-D, CD34, SLAM,
25 eolakin-2, IL-8, MIP-1d, MCP-4, MDC, midkine, TARC, Flt-3
ligand, G-CSF, GM-CSF, c-kit ligand, leptin, oncostatin
M, osteopontin, PP14, SARP-1, follistatin, IGF binding
protein, IGF binding protein, IGF binding protein, Epo R,
G-CSF-R, IL-11 Ra, IL-15 Ra, eNOS, MMP-12, inhibin A (a
30 subunit), 4-1BB, CD30, FasL, TRAIL R2, GM-CSF Rb, IFN-a/b
Rb, IGF-I R, c-kit, M-CSF R, FGF acidic, FGF-3, FGF-4,
FGF R2, FGF R3, integrin-a5, IL-2, IL-3, IL-4, IL-5, IL-
6, IL-7, IL-10, IL-11, IL-1 RII, IL-2 Ra, IL-2 Rb, IL-

2Rg, GADPH; and allelic variants or mutants thereof, wherein said gene products are involved with (a) specific allergic disease(s) or a predispositon therefor to be diagnosed, and wherein said receptors are capable of specifically binding ligands selected from the group consisting of mRNA, oligonucleotides, cDNAs, and proteins or functional fragments thereof,

16. Diagnostic tool according to claim 15, wherein said gene products are selected from the group consisting of EpCAM, DNAM, Endoglin, Flt-3/Flk-2 R, c-met, MPL R, MSP R, IL-18

R, Tie-2, fractalkine, HCC-4, I-309, Agouti-related transcript, PREF-1, SARP-3, Urokinase R, MMP-15, EBAF, TRAIL R2, amphiregulin, betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, neuropilin-1, NGF R, GADPH; and allelic variants or mutants thereof.

20 17. Diagnostic tool according to claim 15 or 16, wherein said immobilized receptors form a patterned array on said surface.

18. Diagnostic tool according to claim 17, wherein said array provides distinct detection areas with at least one receptor immobilized within each such area being representative for the presence, of or predisposition for (a) allergic disease(s).

30 19. Diagnostic tool according to any of claims 15 to 18, wherein the allergic disease or predisposition to be diagnosed is allergic asthma, and wherein the immobilized

receptors specifically represent at least one gene coding for at least one gene product selected from the group consisting of Endoglin, Flt-3/Flk-2 R, c-met, MPL R, IL-18 R, Fractalkine, HCC-4, I-309, PREF-1, SARP-3, Urokinase R, EBAF, Amphiregulin, Betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, BDNF, CNTF, GDNF, GFR α 1, GFR α 2, Neuropilin-1, NGF R, GADPH, and allelic variants or mutants thereof.

10

20. Diagnostic tool according to any of claims 15 to 18, wherein the allergic disease or predisposition to be diagnosed is atopic dermatitis, and wherein the immobilized receptors specifically represent at least one gene coding for at least one gene product selected from the group consisting of EpCAM, DNAM, Flt-3/Flk-2 R, c-met, MSP R, IL-18 R, Tie-2, HCC-4, I-309, Agouti-Related Transcript, SARP-3, Urokinase R, MMP-15, EBAF, Trail R2, Amphiregulin, Betacellulin, Cripto, erbB1, erbB3, Ephrin-A1, Ephrin-A2, EphA1, EphA2, EphA3, EphA4, EphB4, EphB6, and allelic variants or mutants thereof.

Figure 1

A)
**not activated,
directly labelled mRNA
from CD4+ T cells**

B)
**activated CD4+ T cells,
mRNA labelled following in
vitro activation**

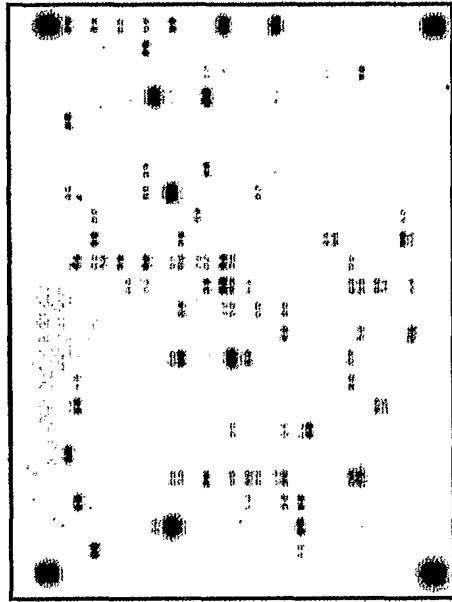
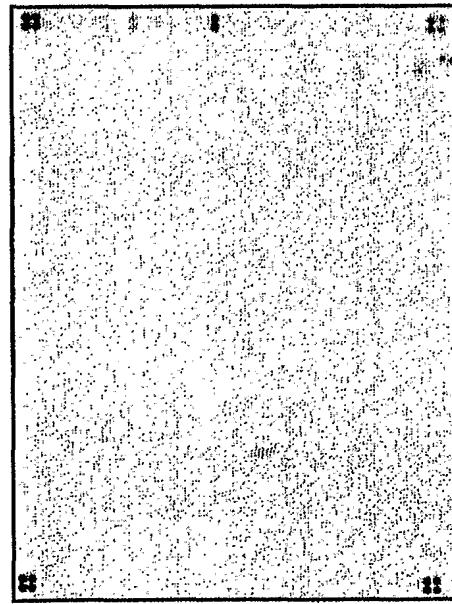


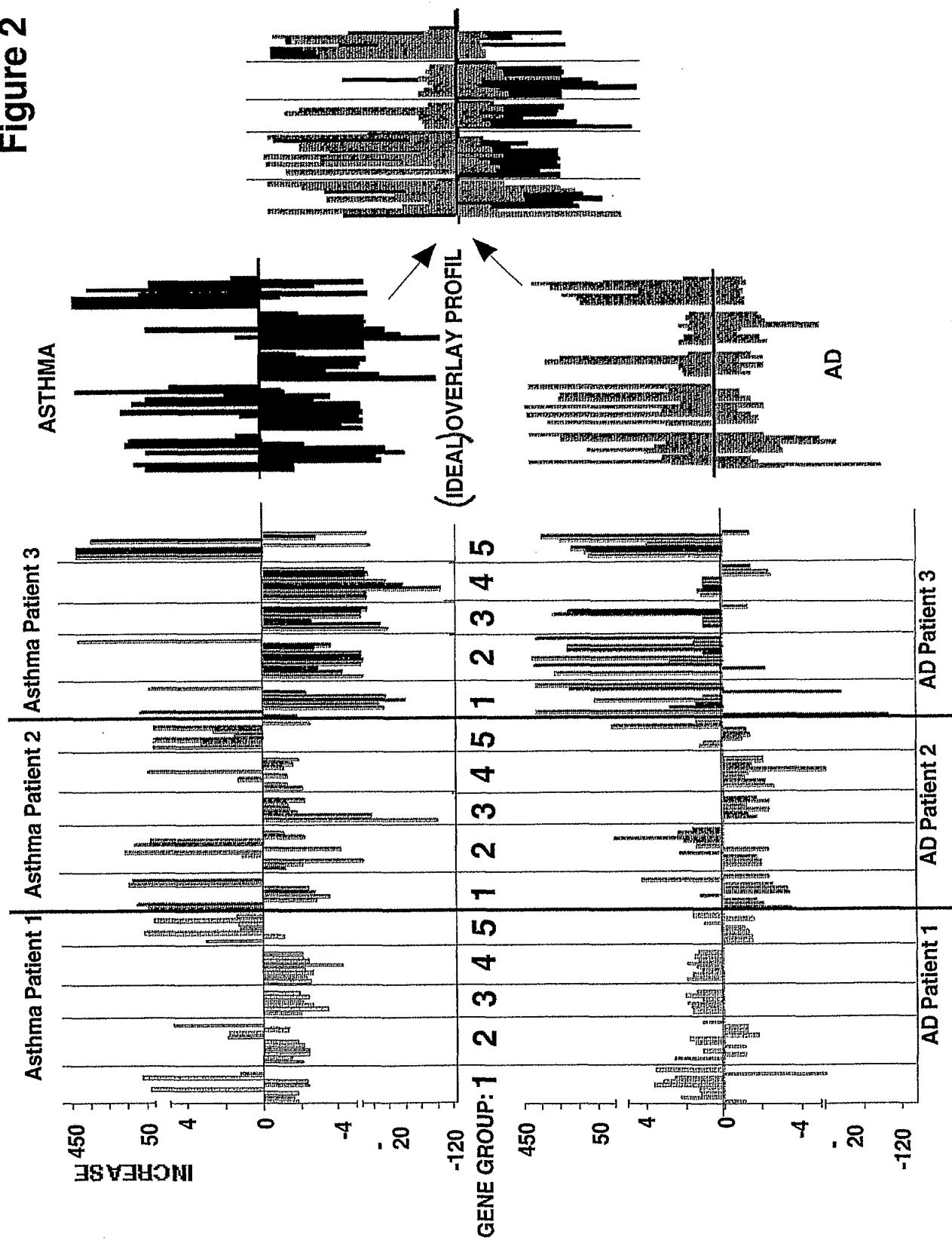
Figure 2

Figure 3**GROUP 1: Surface activation marker**

1. EpCAM
2. DNAM
3. Endoglin
4. Flt-3/FI κ -2 R
5. c-met
6. MPL R
7. MSP R
8. IL-18 R
9. Tie-2

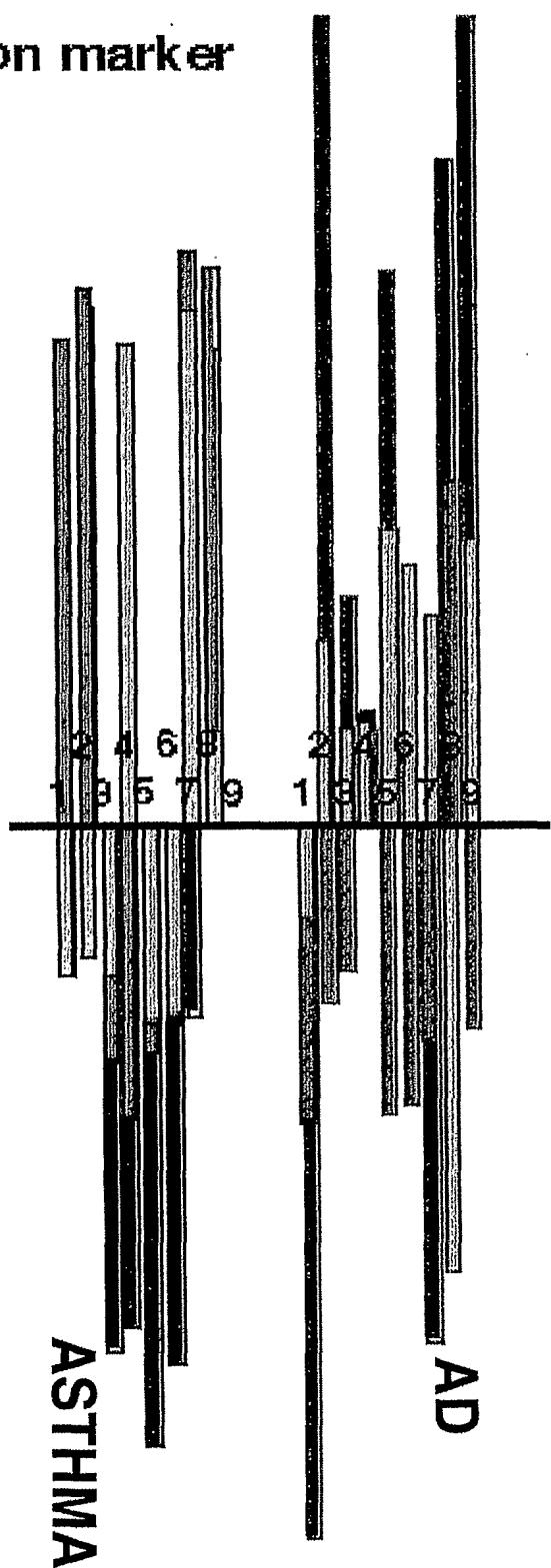


Figure 4
Group 2: Lymphoid cytokine network

1. Fractalkine
2. HCC-4
3. I-309
4. Agouti-Related Transcript
5. PREF-1
6. SARP-3
7. Urokinase R
8. MMP-15
9. EBAF
10. TRAIL R2

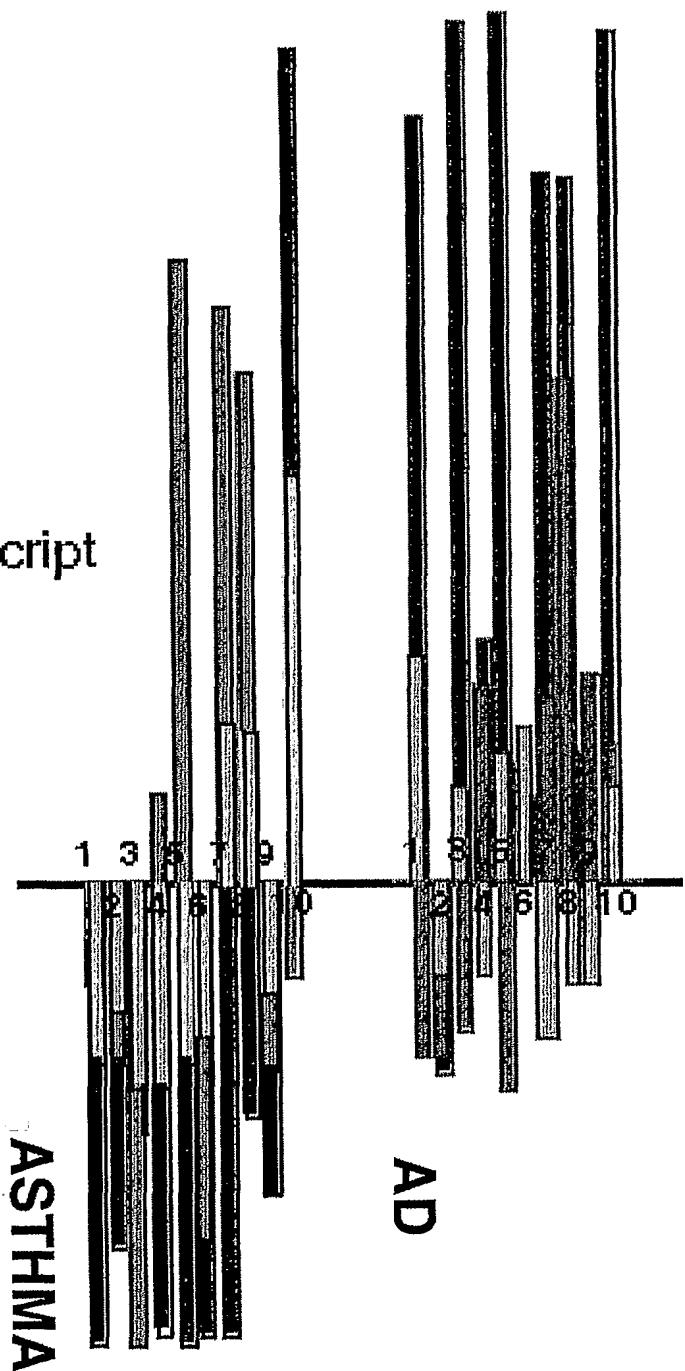


Figure 5**Group 3: Tissue modulators**

1. Amphiregulin
2. Betacellulin
3. Cripto
4. erbB1
5. erbB3
6. TGF- α

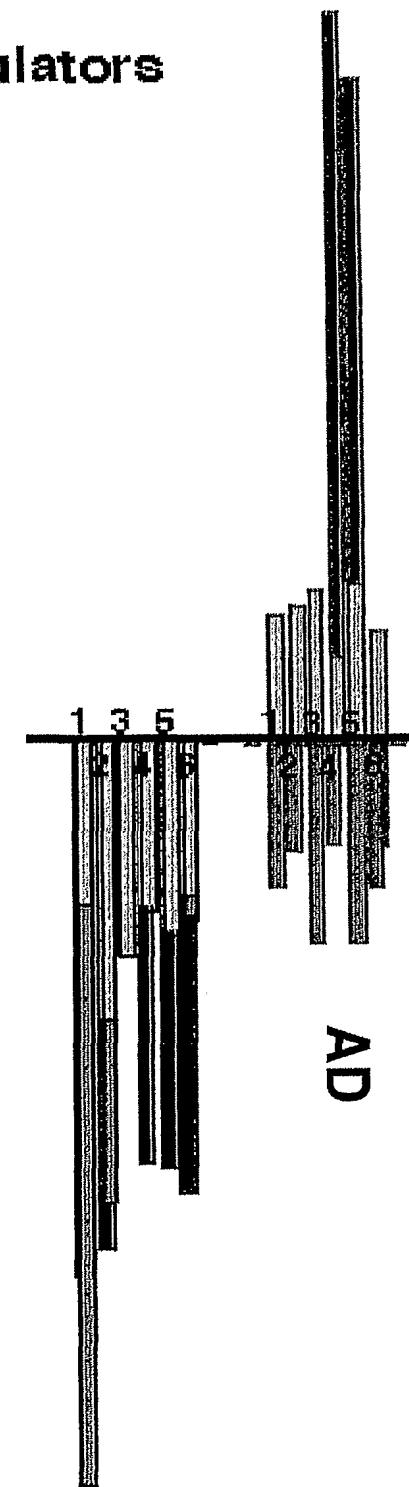


Figure 6
Group 4: neuro-inhibition

1. Ephrin-A1
2. Ephrin-A2
3. EphA1
4. EphA2
5. EphA3
6. EphA4
7. EphB4
8. EphB6

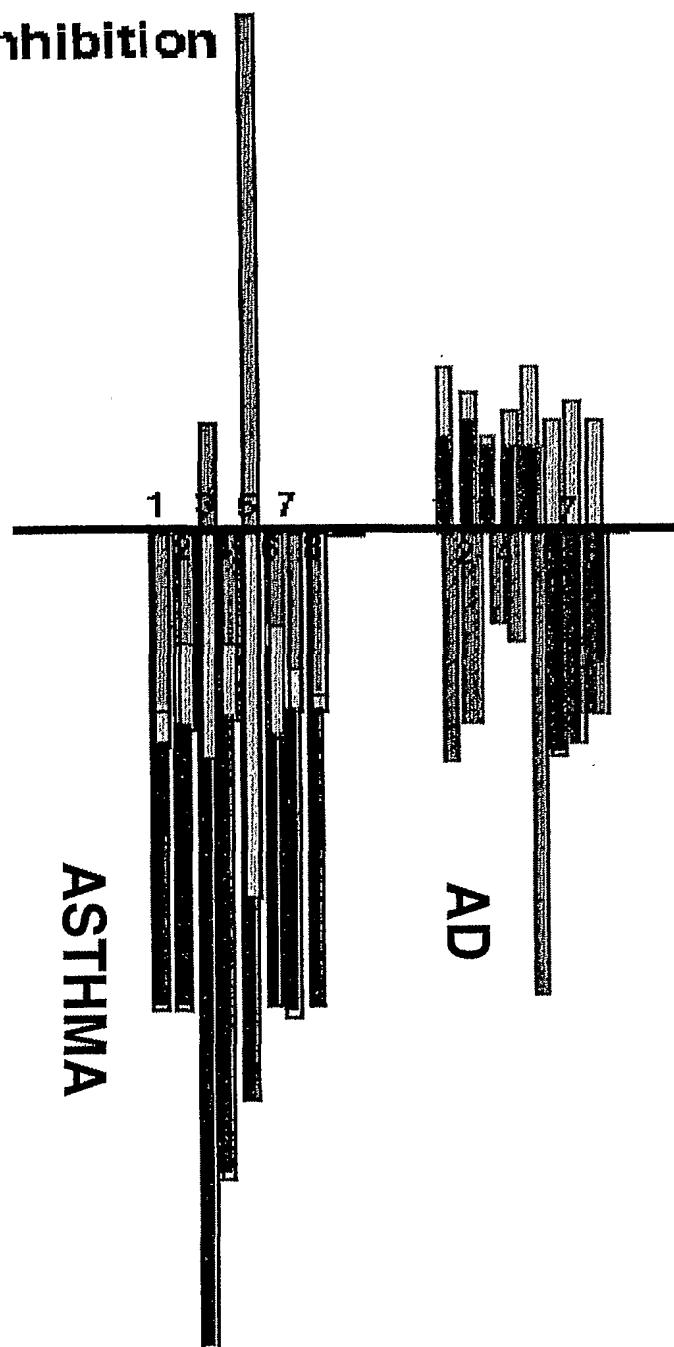


Figure 7
Group 5: Neurotroph

1. CNTF
2. GDNF
3. GFRa1
4. GFRa2
5. Neuropilin-1
6. NGF R

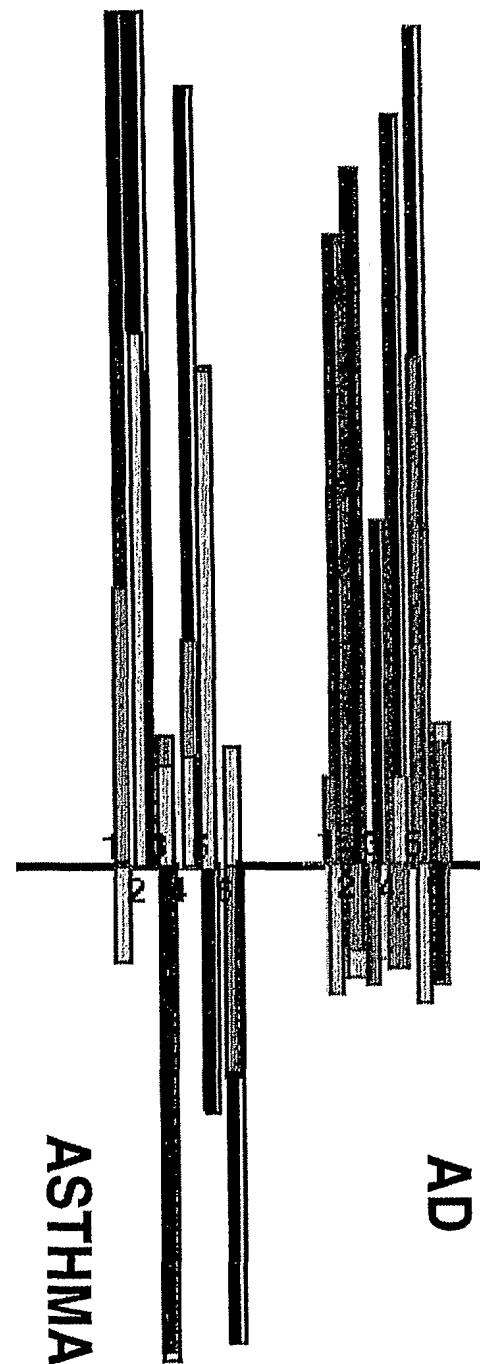


Figure 8

Gene Molecule Family

GROUP 1: Surface activation marker

Gene	Molecule Family	Startposi	length
GROUP 1: Surface activation marker			
EpCAM	Adhesion Molecule	1259	40
DNAM	Cell Surface Protein	2369	40
Endoglin	Cell Surface Protein	1878	40
Fit-3/Fik-2 R	Cytokine Receptor	decreased in Asthma, slight increase in AD	2367
c-met	Cytokine Receptor	decreased in Asthma, strong increase in AD	4305
MPL R	Cytokine Receptor	decreased in Asthma	1854
MSPR	Cytokine Receptor	decrease in AD	3950
IL-18 R	Interleukin Receptor	increased in Asthma & AD	2947
Tie-2	Angiogenic Factor	unchanged in Asthma, rather increased in AD	2618

Group 2: Lymphoid cytokine network

Fractalkine	Chemokine	rather unchanged-decreased in AD, decrease in Asthma	216	40
HCC-4	Chemokine	decrease in Asthma, increase in AD	988	40
I-309	Chemokine	decrease in Asthma & AD	427	40
Agouti-Related Transcript	Cytokine	increased in AD	720	40
PREF-1	Cytokine	decreased in Asthma	1395	40
SARP-3	Cytokine	decreased in Asthma, increased in AD	1108	40
Urokinase R	Protease or Related Factor	slightly increased in AD, varying in Asthma	120	40
MMP-15	Protease or Related Factor	increased in AD	559	40
EBAF	TGF β Superfamily	decreased in Asthma, increased in AD	1847	42
TRAIL R2	TNF Superfamily	increased in AD	2922	40

Group 3: Tissue modulators

Amphiregulin	Epidermal Growth Factor	decreased in Asthma, slightly increased in AD	907	40
Betacellulin	Epidermal Growth Factor	decreased in Asthma, slightly increased in AD	1029	38
Cripto	Epidermal Growth Factor	decreased in Asthma, slightly increased in AD	1034	39
erbB1	Epidermal Growth Factor	decreased in Asthma, slightly increased in AD	4253	40
erbB3	Epidermal Growth Factor	decreased in Asthma, slightly increased in AD	4445	40

TGF- α	Epidermal Growth Factor	decreased in Asthma, slightly increased in AD	692	40
Group 4: neuro-inhibition?				
Ephrin-A1	Ephrin	decreased in Asthma, tendency for increase in AD	929	40
Ephrin-A2	Ephrin	decreased in Asthma, tendency for increase in AD	197	40
EphA1	Ephrin Receptor	decreased in Asthma, tendency for increase in AD	2598	40
EphA2	Ephrin Receptor	decreased in Asthma, tendency for increase in AD	2920	40
EphA3	Ephrin Receptor	decreased in Asthma, tendency for increase in AD	2058	40
EphA4	Ephrin Receptor	decreased in Asthma, tendency for decrease in AD	2443	42
EphB4	Ephrin Receptor	decreased in Asthma, tendency for decrease in AD	3163	40
EphB6	Ephrin Receptor	decreased in Asthma, tendency for decrease in AD	3276	41

CNTF	Neurotrophic Factor	increased in Asthma	682	42
GDNF	Neurotrophic Factor	increased in Asthma	379	40
GFR α 1	Neurotrophic Factor	increased in Asthma	942	40
GFR α 2	Neurotrophic Factor	increased in Asthma	438	40
Neuropilin-1	Neurotrophic Factor	increased in Asthma	2490	40
NGF R	Neurotrophic Factor	increased in Asthma	2885	40

Sequence
AGAAGTATGACACAGCCTAAAGATCATCAGCAATGCCTCC

GADPH
housekeeping gene

Sequence
516 40

Accession	Origin
X13425	Human mRNA for pancreatic carcinoma marker GA733-1.
U56102	Human adhesion molecule DNAM-1 mRNA, complete cds.
X72012	H.sapiens end mRNA for endoglin.
U02687	Human growth factor receptor tyrosine kinase (STK-1) mRNA, complete cds.
X54559	H.sapiens met proto-oncogene mRNA.
M90102	Human (clones 15, 39, 41) c-myeloproliferative leukemia virus type P (c-mpl-P) mRNA, complete cds.
X70040	H.sapiens RON mRNA for tyrosine kinase.
U43672	Human putative transmembrane receptor IL-1Rrp mRNA, complete cds.
U06139	Homo sapiens receptor protein-tyrosine kinase (TEK) mRNA, complete cds.
U91835	Human CX3C chemokine precursor, mRNA, alternatively spliced, complete cds.
U91746	Homo sapiens IL-10-inducible chemokine (HCC-4) mRNA, complete cds.
M57502	Human secreted protein (I-309) mRNA, complete cds.
U88063	Human Agouti related protein (Art) mRNA, complete cds.
U15979	Human (dlk) mRNA, complete cds.
AF017988	Homo sapiens secreted apoptosis related protein 3 (SARP3) mRNA, complete cds.
Z46797	H.sapiens gene for urokinase receptor (partial).
D86331	Human MT2-MMP gene for matrix metalloprotein, complete cds.
U81523	Human endometrial bleeding associated factor mRNA, complete cds.
AF016266	Homo sapiens TRAIL receptor 2 mRNA, complete cds.
M30704	Human amphiregulin (AR) mRNA, complete cds, clones lambda-AR1 and lambda-AR2.
NM_001729	Homo sapiens betacellulin (BTC) mRNA.
X14253	Human mRNA for cripto protein.
X00588	Human mRNA for precursor of epidermal growth factor receptor.
M29366	Human epidermal growth factor receptor (ERBB3) mRNA, complete cds.

M31172	Human transforming growth factor-alpha mRNA, complete cds.
M57730	Human B61 mRNA, complete cds.
AJ007292	Homo sapiens mRNA for ephrin-A2.
M18391	Human tyrosine kinase receptor (eph) mRNA, complete cds.
M59371	Human protein tyrosine kinase mRNA, complete cds.
M83941	Human receptor tyrosine kinase (HEK) mRNA, complete cds.
I36645	Homo sapiens receptor protein-tyrosine kinase (HEK8) mRNA, complete cds.
U07695	Human tyrosine kinase (HTK) mRNA, complete cds.
D83492	Homo sapiens mRNA for Eph-family protein, complete cds.
X60542	Human CNTF gene for ciliary neurotrophic factor.
L19063	Human glial-derived neurotrophic factor gene, complete cds.
U97144	Homo sapiens RET ligand 1 (RETL1) mRNA, complete cds.
AF002700	Homo sapiens GDNF family receptor alpha 2 (GFRalpha2) mRNA, complete cds.
AF018956	Homo sapiens neuropilin mRNA, complete cds.
M14764	Human nerve growth factor receptor mRNA, complete cds.

Gene Name	Gene Group	Accession Number	Gene Description
EpCAM	Adhesion Molecule	X13425	Human mRNA for pancreatic carcinoma marker GA733-1.
ICAM-1	Adhesion Molecule	J03132	Human intercellular adhesion molecule-1 (ICAM-1) mRNA, complete cds.
Angiogenin	Angiogenic Factor	M11567	Human angiogenin gene, complete cds, and three Alu repetitive sequences.
Tie-2	Angiogenic Factor	L06139	Homo sapiens receptor protein-tyrosine kinase (TEK) mRNA, complete cds.
VEGF	Angiogenic Factor	X62568	H sapiens vegf gene for vascular endothelial growth factor.
VEGF-B	Angiogenic Factor	U43368	Human VEGF related factor isoform VRF186 precursor (VRF) mRNA, complete cds.
VEGF-D	Angiogenic Factor	D89630	Homo sapiens mRNA for VEGF-D, complete cds.
CD34	Cell Surface Protein	M81104	Human CD34 mRNA, complete cds.
DNAM	Cell Surface Protein	U56102	Human adhesion molecule DNAM-1 mRNA, complete cds.
Endoglin	Cell Surface Protein	X72012	H.sapiens end mRNA for endoglin.
SLAM	Cell Surface Protein	U33017	Human signaling lymphocytic activation molecule (SLAM) mRNA, complete cds.
Eotaxin-2	Chemokine	NM_002991	Homo sapiens small inducible cytokine subfamily A (Cys-Cys), member 24 (SCYA24) mRNA.
Fractalkine	Chemokine	U91835	Human CX3C chemokine precursor, mRNA, alternatively spliced, complete cds.
HCC-4	Chemokine	U91746	Homo sapiens IL-10-inducible chemokine (HCC-4) mRNA, complete cds.
I-309	Chemokine	M57502	Human secreted protein (I-309) mRNA, complete cds.
IL-8	Chemokine	Y00787	Human mRNA for MDNCF (monocyte-derived neutrophil chemoattractant factor).
MIP-1d	Chemokine	AF031587	Homo sapiens MIP-1 delta mRNA, complete cds.
MCP-4	Chemokine	U46767	Human monocyte chemoattractant protein-4 precursor (MCP-4) mRNA, complete cds.
MDC	Chemokine	U83171	Human macrophage-derived chemokine precursor (MDC) mRNA, complete cds.

Mdkine	Neurotrophic Factor	M69148	Human midkine mRNA, complete cds.
TARC	Chemokine	D43767	Human mRNA for chemokine, complete cds.
Agouti-Related Transcript	Cytokine	U88063	Human Agouti related protein (Art) mRNA, complete cds.
Flt-3 Ligand	Cytokine	U04806	Human FLT3/FLK2 ligand mRNA, complete cds.
G-CSF	Cytokine	X03438	Human mRNA for granulocyte colony-stimulating factor (G-CSF).
GM-CSF	Cytokine	M10663	Human T-cell granulocyte-macrophage colony stimulating factor (GM-CSF) mRNA.
c-kit Ligand	Cytokine	M59964	Human stem cell factor mRNA, complete cds.
Leptin	Cytokine	U18915	Human obese (ob) mRNA, complete cds.
Oncostatin M	Cytokine	AH001516	Human oncostatin M gene, exons 1, 2 and 3.
Osteopontin	Cytokine	J04765	Human osteopontin mRNA, complete cds.
PP14	Cytokine	J04129	Human placental protein 14 (PP14) mRNA, complete cds.
PREF-1	Cytokine	U15979	Human (dlk) mRNA, complete cds.
SARP-1	Cytokine	AF017986	Homo sapiens secreted apoptosis related protein 1 (SARP1) mRNA, partial cds.
Follistatin	Binding Protein	M19480	Human follistatin gene, exons 1-5.
IGF Binding Protein 1	Binding Protein	M31145	Human insulin-like growth factor binding protein mRNA, complete cds.
IGF Binding Protein 5	Binding Protein	M65062	Human insulin-like growth factor binding protein 5 (IGFBP-5) mRNA, complete cds.
IGF Binding Protein 8	Binding Protein	X78947	H.sapiens mRNA for connective tissue growth factor.
Epo R	Cytokine Receptor	M60459	Human erythropoietin receptor mRNA, complete cds.
Flt-3/Flk-2 R	Cytokine Receptor	U02687	Human growth factor receptor tyrosine kinase (STK-1) mRNA, complete cds.
G-CSF R	Cytokine Receptor	M59818	Human granulocyte colony-stimulating factor receptor (G-CSFR-1) mRNA, complete cds.

GM-CSF Rb	Cytokine Receptor	M59941	Human GM-CSF receptor beta chain mRNA, complete cds.
IFN-a/b Rb	Cytokine Receptor	U29584	Human interferon alpha-beta receptor, beta subunit long form mRNA, complete cds.
IGF-I R	Cytokine Receptor	X04434	Human mRNA for insulin-like growth factor I receptor.
c-kit	Cytokine Receptor	X06182	Human c-kit proto-oncogene mRNA.
c-met	Cytokine Receptor	X54559	H.sapiens met proto-oncogene mRNA.
M-CSF R	Cytokine Receptor	X03663	c-fms oncogene; fms oncogene; glycoprotein; membrane protein; proto-oncogene; signal peptide.
MPL R	Cytokine Receptor	M90102	Human (clones 15, 39, 41) c-myeloproliferative leukemia virus type P (c-mpl-P) mRNA, complete cds.
MSP R	Cytokine Receptor	X70040	H.sapiens RON mRNA for tyrosine kinase.
Amphiregulin	Epidermal Growth Factor	M30704	Human amphiregulin (AR) mRNA, complete cds, clones lambda-AR1 and lambda-AR2.
Betacellulin	Epidermal Growth Factor	NM_00172	Homo sapiens betacellulin (BTC) mRNA.
Cripto	Epidermal Growth Factor	X14253	Human mRNA for cripto protein.
erbB1	Epidermal Growth Factor	X00588	Human mRNA for precursor of epidermal growth factor receptor.
erbB3	Epidermal Growth Factor	M29366	Human epidermal growth factor receptor (ERBB3) mRNA, complete cds.

TGF-a	Epidermal Growth Factor	M31172	Human transforming growth factor-alpha mRNA, complete cds.
Ephrin-A1	Ephrin	M57730	Human B61 mRNA, complete cds.
Ephrin-A2	Ephrin	AJ007292	Homo sapiens mRNA for ephrin-A2.
EphA1	Ephrin Receptor	M18391	Human tyrosine kinase receptor (eph) mRNA, complete cds.
EphA2	Ephrin Receptor	M59371	Human protein tyrosine kinase mRNA, complete cds.
EphA3	Ephrin Receptor	M83941	Human receptor tyrosine kinase (HEK) mRNA, complete cds.
EphA4	Ephrin Receptor	L36645	Homo sapiens receptor protein-tyrosine kinase (HEK8) mRNA, complete cds.
EphB4	Ephrin Receptor	UO7695	Human tyrosine kinase (HTK) mRNA, complete cds.
EphB6	Ephrin Receptor	D83492	Homo sapiens mRNA for Eph-family protein, complete cds.
FGF acidic	Fibroblast Growth Factor Family	M13361	Human beta-endothelial cell growth factor (ECGF-beta) mRNA, complete cds.
FGF-3	Fibroblast Growth Factor Family	X14445	Human int-2 proto-oncogene.
FGF-4	Fibroblast Growth Factor Family	M17446	Human Kaposi's sarcoma oncogene fibroblast growth factor mRNA, complete cds.
FGF R2	Fibroblast Growth Factor Family	U11814	Human soluble keratinocyte growth factor receptor mRNA, alternatively spliced complete cds.
FGF R3	Fibroblast Growth Factor Family	M58051	Human fibroblast growth factor receptor (FGFR3) mRNA, complete cds.
Integrin-a5	Integrin	X06256	Human mRNA for integrin alpha 5 subunit.
IL-2	Interleukin	V00564	Human mRNA encoding interleukin-2 (IL-2) a lymphocyte regulatory molecule.
IL-3	Interleukin	M14743	Human interleukin 3 (IL-3) mRNA, complete cds.
IL-4	Interleukin	M13982	Human interleukin 4 (IL-4) mRNA, complete cds.
IL-5	Interleukin	X04688	Human mRNA for T-cell replacing factor (interleukin-5).
IL-6	Interleukin	M14584	Human interleukin 6 mRNA, complete cds.
IL-7	Interleukin	J04156	Human interleukin 7 (IL-7) mRNA, complete cds.
IL-10	Interleukin	M57627	Human interleukin 10 (IL10) mRNA, complete cds.
IL-11	Interleukin	M57765	Human interleukin 11 mRNA, complete cds.

IL-1 RI	Interleukin Receptor	U64094	Human soluble type II interleukin-1 receptor mRNA, complete cds.
IL-2 Ra	Interleukin Receptor	X01057	Human mRNA for interleukin-2 receptor.
IL-2 Rb	Interleukin Receptor	M26062	Human interleukin 2 receptor beta chain (p70-75) mRNA, complete cds.
IL-2 Rg	Interleukin Receptor	D11086	Human mRNA for interleukin 2 receptor gamma chain.

IL-11 Ra	Interleukin Receptor	Z38102	H.sapiens mRNA for interleukin-11 receptor.
IL-15 Ra	Interleukin Receptor	U31628	Human interleukin-15 receptor alpha chain precursor (IL15RA) mRNA, complete cds.
IL-18 R	Interleukin Receptor	U43672	Human putative transmembrane receptor IL-1Rrp mRNA, complete cds.
BDNF	Neurotrophic Factor	X91251	H.sapiens mRNA for BDNF protein.
CNTF	Neurotrophic Factor	X60542	Human CNTF gene for ciliary neurotrophic factor.
GDNF	Neurotrophic Factor	L19063	Human glial-derived neurotrophic factor gene, complete cds.
GFRa2	Neurotrophic Factor	AF002700	Homo sapiens GDNF family receptor alpha 2 (GFRalpha2) mRNA, complete cds.
Neuropilin-1	Neurotrophic Factor	AF018956	Homo sapiens neuropilin mRNA, complete cds.
NGF R	Neurotrophic Factor	M14764	Human nerve growth factor receptor mRNA, complete cds.
eNOS	NO Metabolism	L26914	Human nitric oxide synthase mRNA, complete cds.

Urokinase R	Protease or Related Factor	Z46797	H.sapiens gene for urokinase receptor (partial).
MMP-12	Protease or Related Factor	L23808	Human metalloproteinase (HME) mRNA, complete cds.
MMP-15	Protease or Related Factor	D86331	Human MT2-MMP gene for matrix metalloprotein, complete cds.
EBAF	TGF b Superfamily	U81523	Human endometrial bleeding associated factor mRNA, complete cds.
Inhibin A (a subunit)	TGF b Superfamily	M13981	Human inhibin A-subunit mRNA, complete cds.

4-1BB	TNF Superfamily	U03397	Human receptor protein 4-1BB mRNA, complete cds.
CD30	TNF Superfamily	M83554	H.sapiens lymphocyte activation antigen CD30 mRNA, complete cds.
FasL	TNF Superfamily	X89102	H.sapiens mRNA for fasligand.
TRAIL_R2	TNF Superfamily	AF016266	Homo sapiens TRAIL receptor 2 mRNA, complete cds.